Legends for supplementary figures

Supplementary figure 1. Caffeine induces autophagy in PC12D and HeLa cells.

A, B. PC12D (A) and HeLa (B) cells treated with 10 mM caffeine were analyzed by immunoblotting with antibodies against LC3 and actin. Densitometry analysis of LC3-II levels relative to actin was performed from three independent experiments. C, D. HeLa cells treated with 10 mM caffeine for 24 hours were analyzed by immunoblotting with antibodies against p62 and actin. Densitometry analysis of p62 levels relative to actin was performed using three independent experiments. E. HeLa cells treated with or without 10 mM caffeine for 24 hours were stained with antibodies against LC3. They were analyzed with confocal microscopy. Bar, 10 μm. F, G. SH-SY5Y cells treated with 10 mM caffeine for 9 hours, followed by treatment with or without 400 nM Bafilomycin A1 for 3 hours, were analyzed by immunoblotting. Densitometry analysis of LC3-II levels relative to actin was performed using three independent experiments. H, I. HeLa cells treated with 25 mM caffeine for 3 hours, followed by treatment with or without 400 nM Bafilomycin A1 for 3 hours, were analyzed by immunoblotting. Densitometry analysis of LC3-II levels relative to actin was performed using three independent experiments. Data are the means of triplicate experiments.Error bars, S.D.; **, $p<0.01$; ***, $p<0.001$

Supplementary figure 2. Caffeine inhibits the mTOR signaling pathway.

A. HeLa cells treated with or without 10 mM caffeine for 24 hours were analyzed by immunoblotting with antibodies against phosphor-Akt (Ser473), Akt and actin. B.C. HeLa cells treated with 10 mM caffeine for various time periods were analyzed by immunoblotting for levels of phosphor- and total p70 ribosomal S6 protein, S6, Akt and actin.

Supplementary figure 3. Rapamycin with caffeine has an additive effects on enhancement of autophagy.

A. SH-SY5Y cells were treated with various concentration of rapamycin for 24 or 48 hours to detect the concentrations enough to induce autophagy. Cell lysates were analyzed with immunoblotting. B, C.

HeLa cells treated with 0.5 μM rapamycin or DMSO along with or without 10 mM caffeine were analyzed by immunoblotting. Densitometry analysis of each protein levels relative to actin was performed form three independent experiments. Error bars, S.D. NS, not significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Supplementary figure 4. Caffeine induces autophagy in a dose-dependent manner.

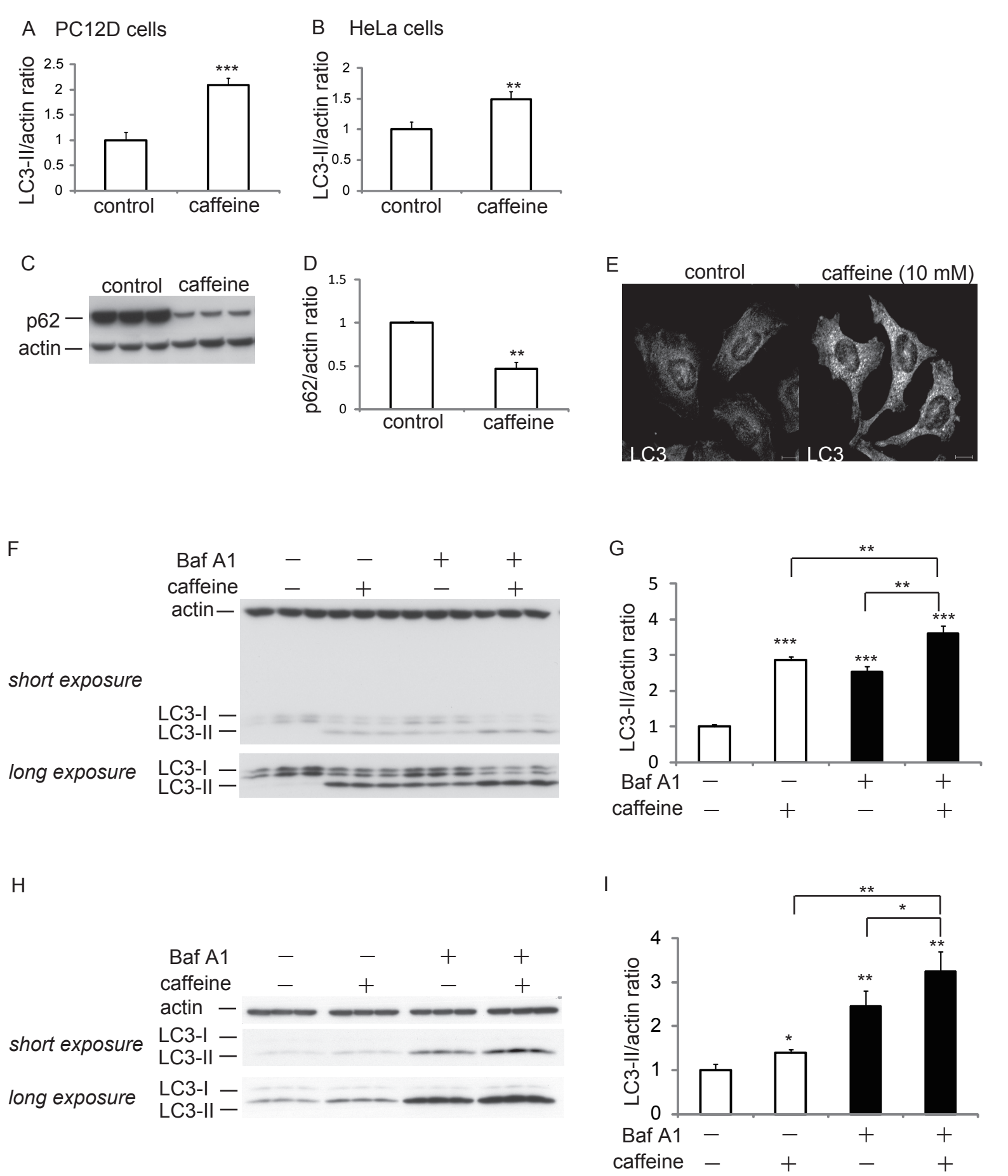
A-E. PC12D cells (A, B), Atg7+/+ mouse embryonic fibroblasts (MEFs) (C, D) and Atg7-/- MEFs (E) treated with various concentrations of caffeine for 24 hours were analyzed by immunoblotting with antibodies against LC3 and actin. Densitometry analysis of LC3-II levels relative to actin was performed from three independent experiments (B, D). Data are the means of triplicate experiments. Error bars, S.D. NS, not significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Supplementary figure 5. Caffeine induces reduction of mitochondrial membrane potentials dependently of autophagy.

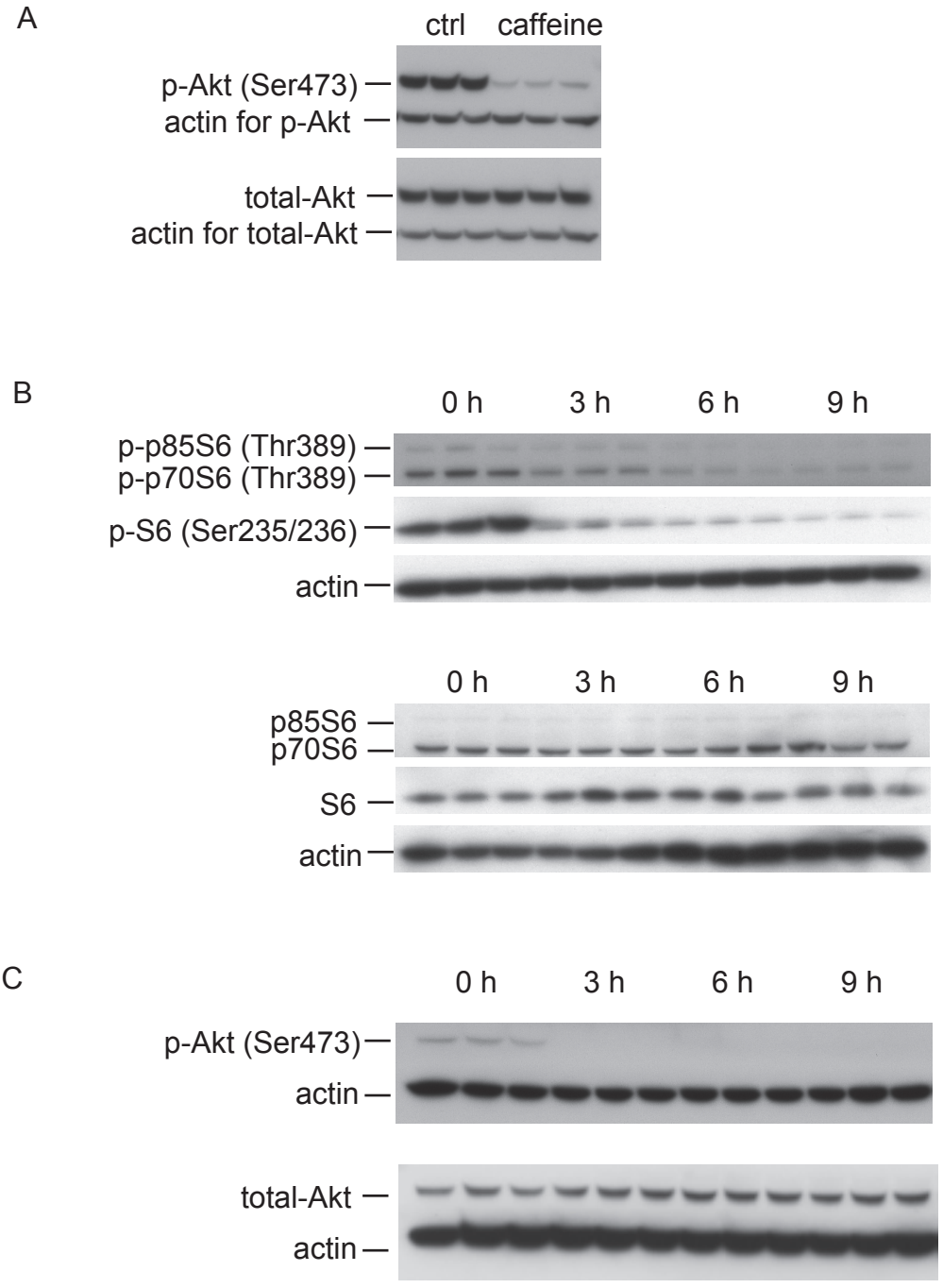
A. Representative FACS data of experiments shown in Fig. 6B (refer to the legends of Fig. 6B). B. Representative FACS data of experiments shown in Fig. 6F (refer to the legends of Fig. 6F).

Supplementary figure 6. Autophagy-deficient cells are more resistant to caffeine-induced apoptosis.

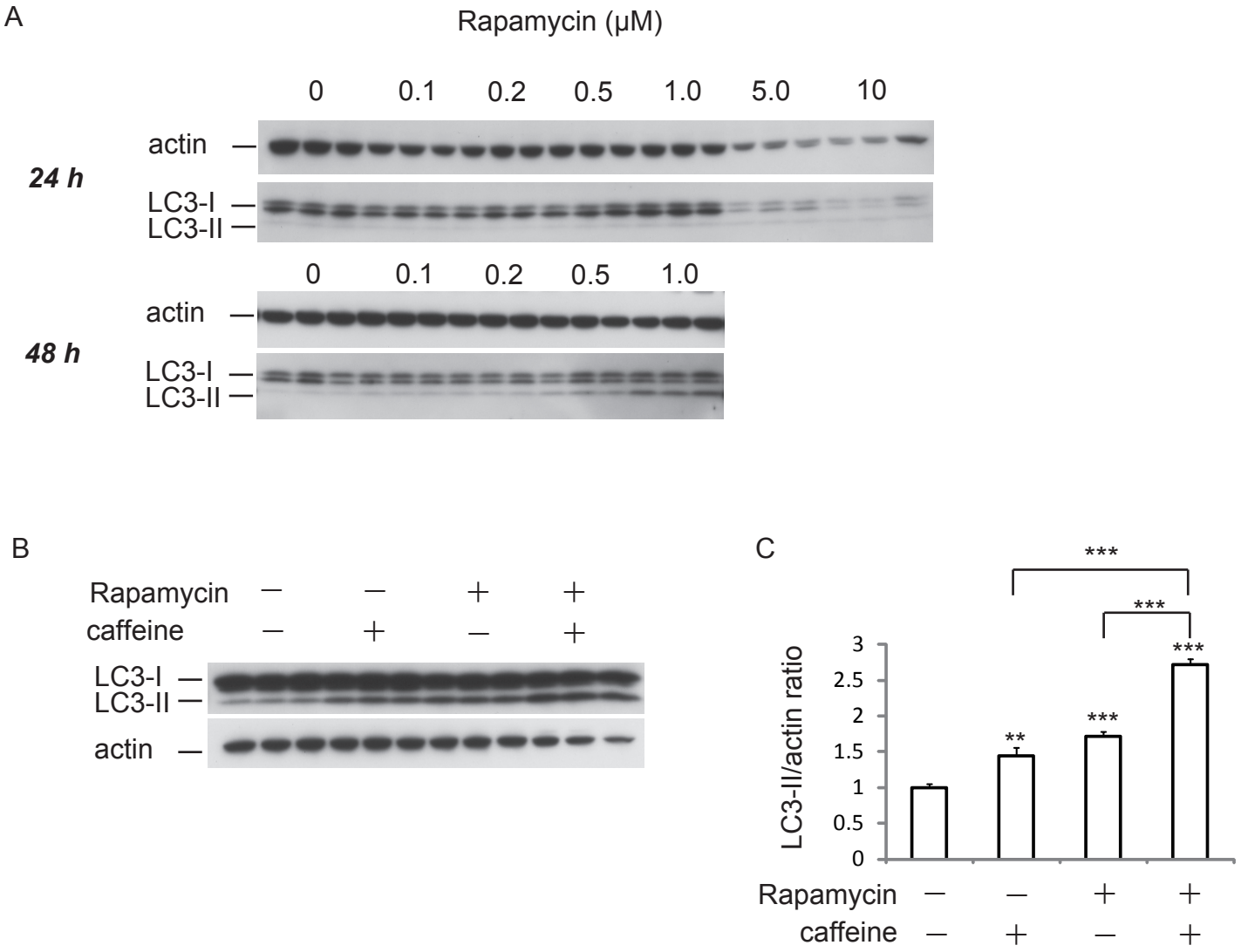
A. The M1 gate demarcate annexin V positive (apoptotic or necrotic cells) population. B. Data are the means of triplicate experiments. Error bars, S.D. NS, not significant; **, $p<0.01$; ***, $p<0.001$



Supplementary figure S1.

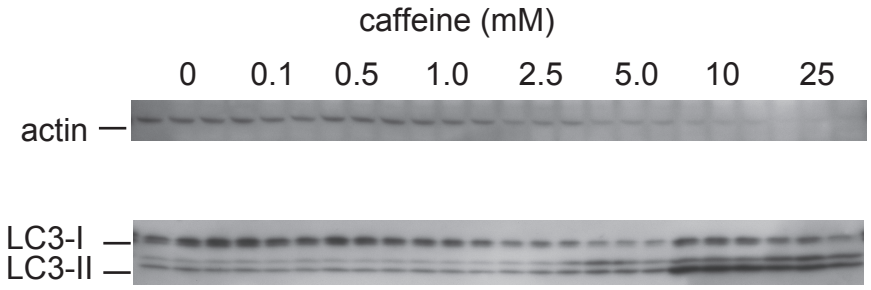


Supplementary figure S2.



Supplementary figure S3.

A PC12D cells



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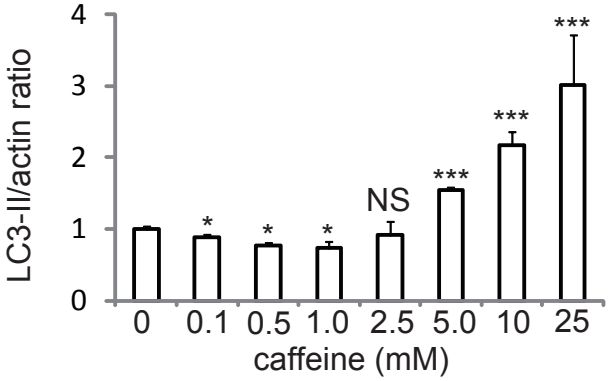
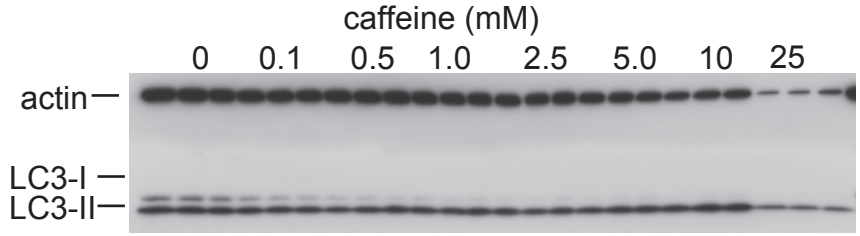


C Atg7 +/+ MEFs



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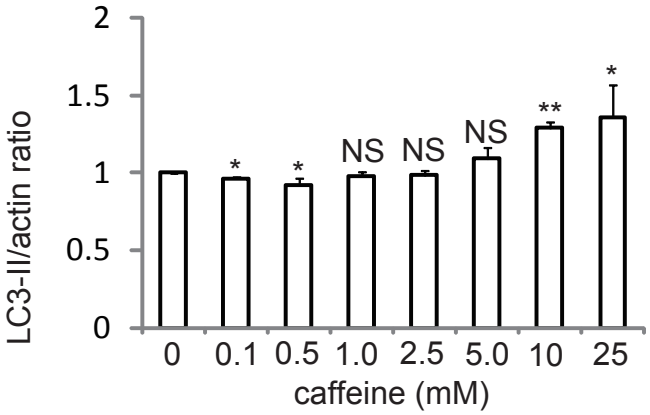

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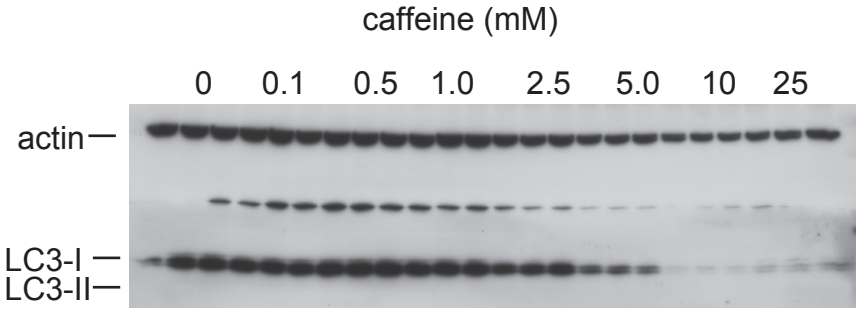
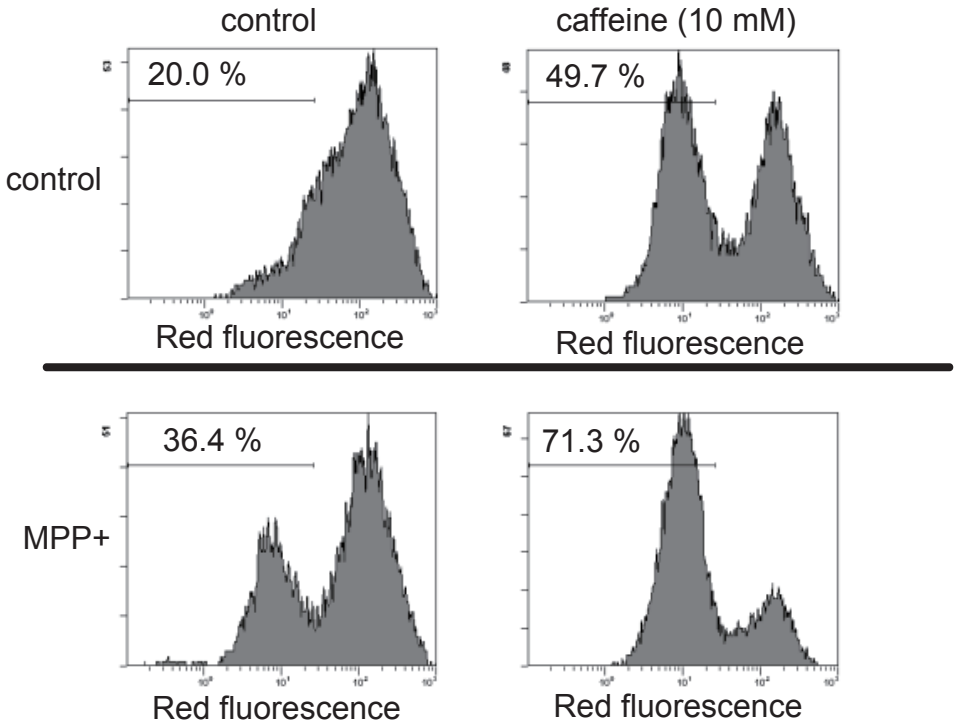


Supplementary figure S4.

A



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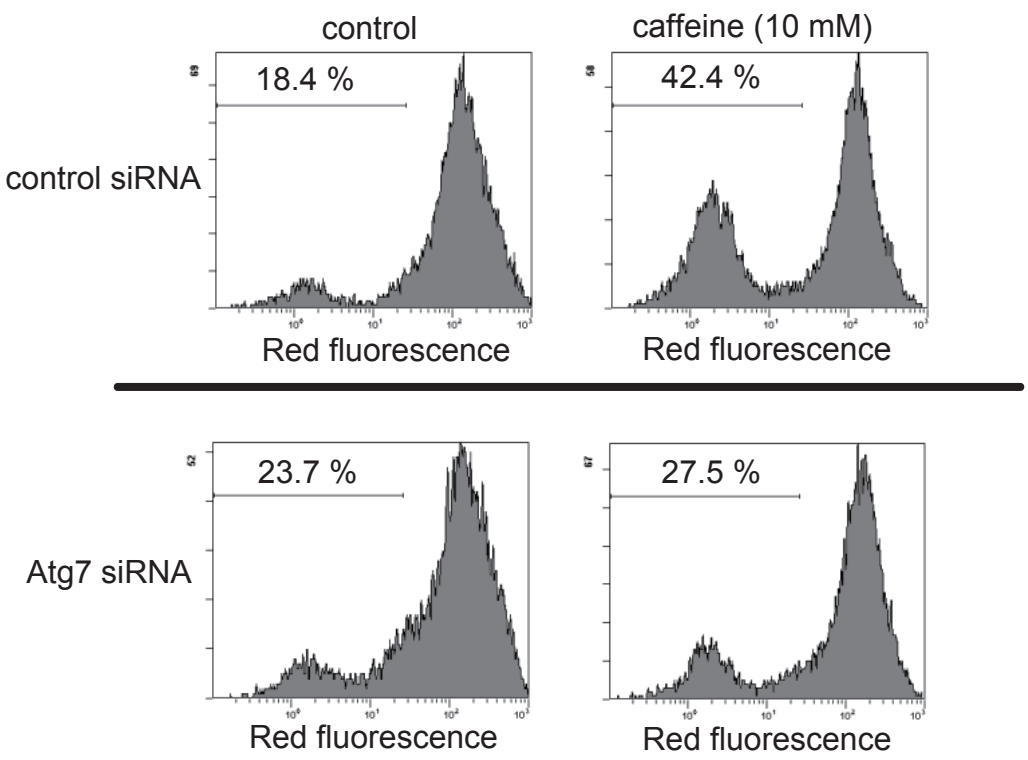

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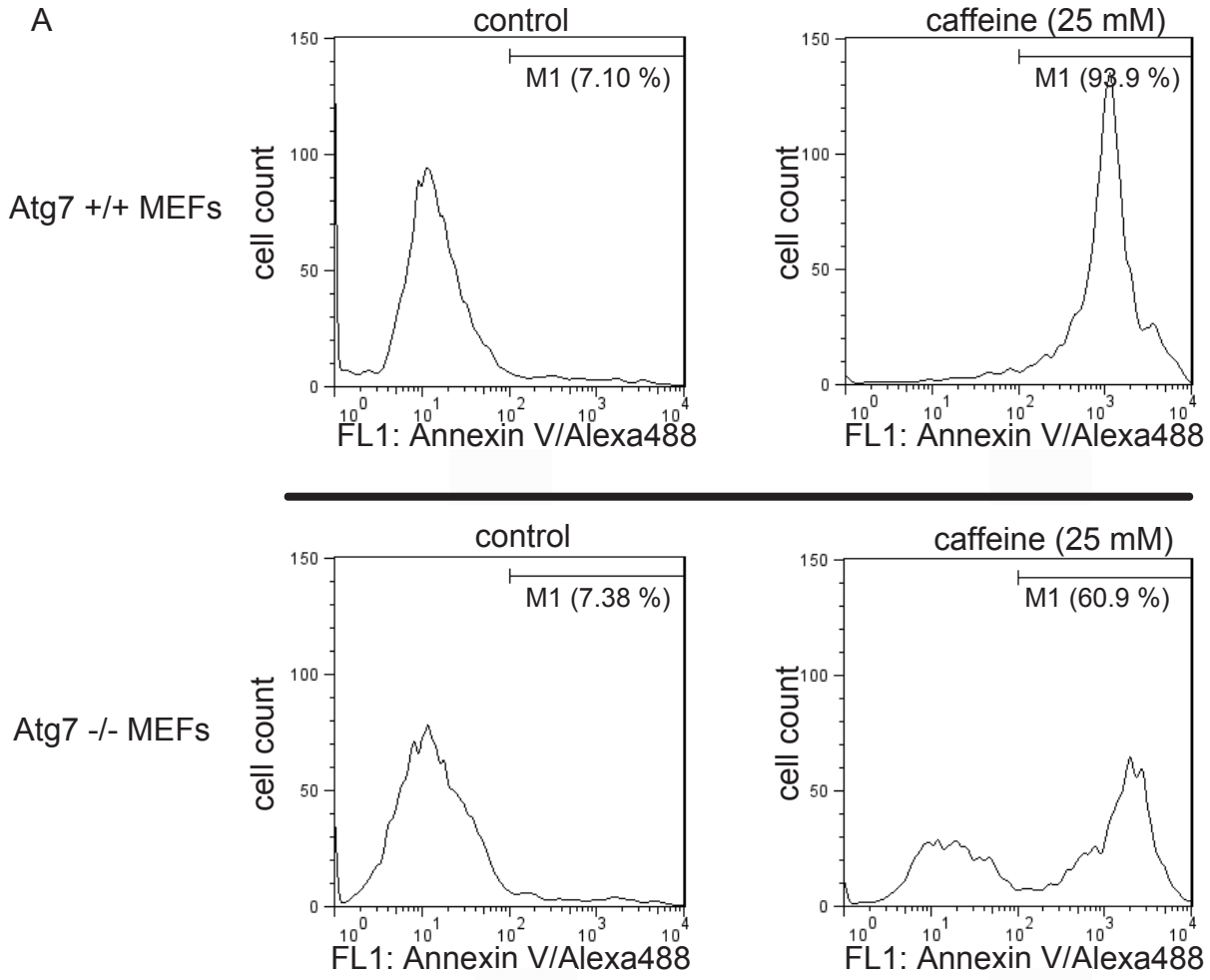

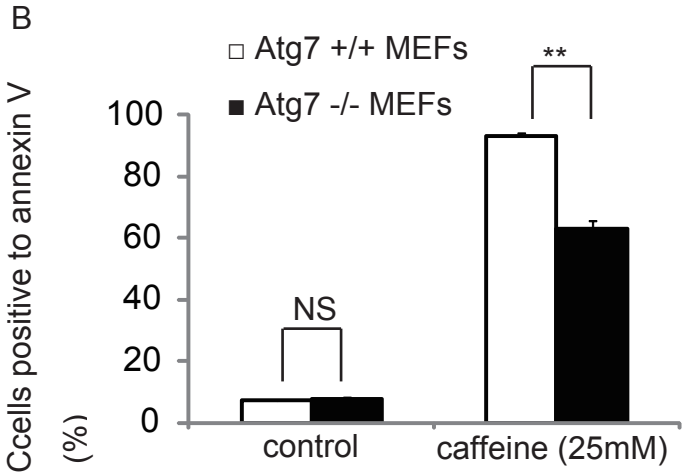

Supplementary figure S6.